Conclusions regarding transposition of Spm in the a_1^{m-1} cultures.

The progeny tests described both in this section and in the last section provide some information regarding stability of location of Spm. It is clear from that that the time of occurrence of transposition of Spm during development, and the frequency of this at any one time, is controlled in some manner. Change in this undergone by an Spm element appeared to be associated with transposition, its behavior in the former location and in the new location often being decidedly different. The altered control of its behavior may be an expression of some modification occurring to Spm during the transposition process, or it may reflect participation in this of the particular locus in the chromosome complement at which Spm is inserted. Up to now, no evidence is available from study of Spm favoring one or the other of these interpretations. As mentioned earlier, evidence favoring the second interpretation was obtained from study of transposition of Ds. Evidence of altered behavior of Spm, following transposition of it, was given by several of the described tests. It was shown that following some transpositions, the relative frequency of occurrence of subsequent transposition of Spm, and the time of this during development, could be strikingly different from that which it expressed at its former location. Cases were cited in which it had been

relatively stable when present at one location but became quite unstable after it had been inserted at a new location. Cases in which the reverse of this occurred, also were cited.

It was shown that when Spm resided at a particular site in chromosome 6 in those plants in culture 6629A and in thier progeny which gave approximately 35 percent recombinants with Y (table 29), it remained relatively stable in this location, the frequency of appearance of progeny plants in which Spm occuppied a new location being low. Nevertheless, transposition of it from this location to a new location was observed and several cases of this were subsequently analyzed (Progeny Tests 20 to 23, figure 2). Among them was one bazaar case involving the behavior of Spm in plant 6666C-7 and its progeny (Progeny Tests 14 to 16, figure 2). In this plant, transposition of Spm occurred at a very high rate. From the type of sterility that was expressed by each of the two testcross ears obtained from this plant, it is suspected that insertion of Spm at some locations in the chromosome complement may result in change in gene action at the site of insertion, and this may cause either lethality for the cell having it at one such site, or it may cause some modification of gene action that alters the capacity of the cell to function normally.

In those cases where its insertion at a new location did not produce such deleterious effects, viable kernels in which Spm occupied a new location were produced. The location and behavior of Spm in the plants derived from each could be analyzed. This was done for five kernels on one of the ears of plant 6666C-7. In the plants derived from each of them Spm occuppied a different location. One transposition had inserted Spm close to Y in chromosome 6 and from this location few subsequent transpositions occurred (Progeny Test 15, figure 2). In contrast, an insertion of it into chromosome 9 resulted in a high frequency of subsequent transpositions, and these occurred both during early and late development of those plants that had it at this location in their zygote nuclei (Progeny Test 16, figure 2). In contrast to this, another case of insertion of Spm into chromosome 9 was followed by subsequent transpositions of it that were confined mainly to the early stages of plant development (Progeny Tests 6 to 13, figure 2).

The mode of examining transposition of Spm did not allow detection of those cases that placed it at a new location in the same chromosome in which it had been residing, when this new location was close to that which it formerly occuppied. Therefore, accurate estimates of rates of transposition of Spm could not be made. Estimates of this may be

formulated only from those detected cases that placed Spm in a new location, either in another chromosome of the complement or at a location in the same chromosome sufficiently distant from its former location to markedly alter the frequency of appearance of recombinants with the gene marker in the chromosome that was used for determining its presence in this chromosome. Even though it is realized that transposition of a controlling element frequently places it at a new location that is not far removed from its former location in the same chromosome, and that in this study estimates of the frequency of occurrence of this could not be made, it was apparent, nevertheless, that differences in rates and times of occurrence of transposition of Spm could be expressed to a striking degree when measured only by those cases of it that were readily detectible.

The test methods used in this study allowed detection of the location of Spm if it had been inserted into chromosomes 3, 5, 6, or 9, and if it resided close enought to an allele of the markers Sh_2, Pr, Y, or Wx to give evidence of linkage of it with any one of them. Cases of its insertion at different locations in chromosomes 5, 6, and 9 were discussed in this section along with the characteristic behavior of Spm at each location in one of these chromosomes. It is probably only a

coincidence that the examined cases of insertion of Spm at different locations in chromosome 6 were followed by a considerable degree of stability of Spm at each of these locations whereas, in contrast, the examined cases of insertion of it in chromosome 9 were followed by high rates of subsequent transposition. It was also noted that in sequential transpositions of Spm no evidence of preferance of one location over another was exhibited. In other words, if Spm were transposed from a known location to a new location, subsequent transposition of it did not tend to return it to the location it formerly occuppied.

Linkage of Spm with a given gene marker, carried either in chromosome 5, 6, or 9, was examined in a number of plants. In some of the tables in this section is entered the percent of the recombinant classes or the percent of one of them. It is obvious from those given in the tables accompanying the descriptions of Progeny Tests 5, 6, 24, and 26, that such percents do not serve as a measure of crossing over between the site of insertion of Spm in the chromosome and the locus of the gene marker. If the rate of late

occurring transposition is high, then many individuals within the recombinant class carry in them a newly transposed Spm element, as discussed earlier (page). Also, the closer is Spm to the marker, the less reliance may be placed on the given percent of recombination in estimating the location of Spm with reference to the marker. Only by means of progeny tests can an accurate estimate of this be made.

In this study, a number of cases were mentioned in which plants having two or more Spm elements appeared in the progeny of ones in which only one Spm was present. An explanation of the probable origin of cells having two Spm elements in them from ones in which only one was present, and the relation of this to cells having no Spm, was discussed earlier (page000). It might be suspected that cells having more than 2 Spm elements in them could arise from successive transpositions of Spm in the progeny of cells in which two were present. This may be the sequence of events that is responsible for the appearance of some cells having more than 2 Spm elements in them but possibly this explanation may not apply to all of them. Cases of similar type were examined by Brink with the controlling element Mp (= Ac) in which he could not relate all of this type to sucdessive transpositions of Mp. For study of transposition, Ac is much more favorable than Spm because change in dose of it leads to readily recognizable

change in phenotypic expression, each dose being correlated with a particular type of change in expression of the gene whose mutation pattern it controls. The dose of Spm is difficult to determine on the basis of phenotypic pattern produced by a_1^{m-1}. A change in this has not effect on the pattern of mutation but it does have an effect on the frequency of appearance of the pale pigmented areas, either in plant or kernel, that arise from loss or inactivation of Spm. The higher the dose of Spm, the less frequent will cells be formed in which Spm is either absent or inactive.

From the discussions given in this and the previous section, it is evident that the number of Spm elements that will appear in individuals of the progeny of one having a known number of them will depend upon the behavior of the particular Spm elements that are present in the parent. Nevertheless, a summary of the relation between the number of Spm elements in a palnt and that in its progeny may be of some interest and, therefore, table 60 was prepared to illustrate this. In its preparation, the Spm number in the part of the plant that produced the first ear on the main stalk was used. Included in the table are the progeny tests that were conduced during the summers of 1952 to 1955. In this table, no consideration is given to the number of plants in which a newly transposed

Spm element was known to be present. The table merely gives the number of Spm elements that were present in the part of the plant that produced the first ear on the main stalk. Tables 61 and 62 illustrate the agreement with and the differences in the number of Spm elements that were present in different parts of the same plant. In these tables, also, no consideration is given to known differences in location of Spm in different parts of one plant. Only Spm number is considered.

In the studies so far reported, no case was detected of complete stability of location of Spm, thatis, in which Spm remained at one location. ~~Only one case has been found in which no evidence has been given so far of its transposition from a known location~~ in chromosome 5 to a new location in the chro osome complement, and it is a ~~case in which the~~ state of Spm different from that which was present in all plants so far ~~considered~~. ~~This state of Spm, symbolized~~ Spm-w because of its weakened capacity to ~~suppress~~ gene action at a_1^{m-1} ~~and~~ a_2^{m-1} ~~and to induce mutation~~ to or towards A_1 or A_2, will be considered in the ~~next part of this report.~~

PART IV

Change in Mode of Action of Spm

1. Spm with reduced action capacity : Spm-w

On some of the ears produced by plants in culture 6629, grown in the summer of 1953, a few kernels appeared that had only one or several dots of A_1 type pigment in a non-pigmented background instead of the many spots of this pigment type that were exhibited by most of the variegated kernels on the ears of these plants. (see photo -) Kernels of the former type were selected from 8 of the ears. (see table 1?) Each of the 37 plants derived from them was pale pigmented, instead of being variegated for a few streaks in a non-pigmented background as if no Spm were present in any one of them. On the testcross ears produced by the majority of these plants, only pale pigmented kernels appeared. On the ears of several of them, however, many kernels showing the same phenotype as that given by the kernel from which the plant arose. (see photo) One of them was plant 6683B-2, which was a_1^{m-1} (state 5719A-1) Sh_2/a_1 sh_2, Pr/Pr, y/y, Wx/wx in constitution. An ear of this plant had been used in a cross with a tester plant that was Y/y and was homozygous for state 5719A-1 a_1^{m-1} and for Sh_2, pr and wx and it had no Spm. This same tester plant had been used in making many crosses during the summer of 1954 and it was known that the a_1^{m-1} in it would respond to Spm by giving the pattern of pigmentes spots shown in photo . On the ear produced by plant 6683B-2, there were both pale and variegated colored kernels but in the variegated class, the number of pigmented spots

(P360 00)

was very low, the majority of kernels having only 1 or 2 such spots. Also, as stated above, plant 6683B-2 was pale pigmented instead of being variegated, as would be expected if Spm were present. The kernels on the testcross ear of plant 6683B-2 were of three types; 169 were uniformly pale pigmented, 118 were colorless except for 1 or several dots of the A_1 type pigment, and 51 were totally colorless. If the last two classes are combined, there is a 1 to 1 ratio of pale colored kernels to those that are colorless, or nearly so. This ratio suggested that in this plants some factor resembling Spm in its action was segregating. It suppressed gene action at a_1^{m-1} in the kernel but not in the plant, and it was much less effective in inducing mutation to or towards A_1 in the kernel. Some of the kernels that had 1 or several small A_1 dots in a colorless background were sown in the summer of 1955 under culture number 6888. Tests conducted with the plants derived from them indicated that the factor responsible for the altered phenotypic expression of a_1^{m-1} had been carried in one of the chromosomes 5 of the parent plant, 6683B-2. It resembled an Spm whose capacity for action had been much weakened and for this reason it was given the symbol Spm-w. Study of this Spm-w extended through 5 generations of plants and these included examination of the effects it produced on four different states of ~~both~~ a_1^{m-1}.

Before these investigations are reported, several other cases will be reviewed briefly in which a modified type of Spm action was detected in the progeny of individual plants in which a fully active Spm was known to be present.

As mentioned above, only a few of the plants derived from kernels having the described modified type of variegation pattern that were grown in the summer of 1954 gave evidence of the presence in them of Spm, either by their appearance or from the phenotypes of the kernels on their testcross ears they produced. At that time, the reason for this was not understood. It was only sometime later that it was learned that one of the types of change in Spm, responsible for the appearance of some of the kernels with only a few A_1 dots in them, may be inactivation of Spm. In plants having a fully active Spm, change from the active to the inactive phase may take place in some cells, and when this is occurring in a kernel, the altered activity may be noted by an altered pattern of variegation within the aleurone layer of the kernel. In plants derived from such kernels, Spm may remain in its inactive phase throught development of the plant. The nature of this type of change in Spm will be described in the section of this report dealing with inactivation of Spm. Change of Spm from fully active to partially active likewise occurs in some cells and the differences in grade of this

may be distinguished. It is these latter changes in Spm that this section will consider.

On none testcross ears produced by the fully variegated plants grown in the summer of 1954, kernels having the above mentioned types of change in Spm were noted. Often, only one to several kernels on an ear exhibited an altered pattern of variegation. On a few ears, however, a large number of them appeared, and this was true of the testcross ear produced by plant 6665E-9 which was a_1^{m-1}(state 5719A-1) Sh_2/a_1 sh_2, Y/y in constitution (see table 18 for origin of plant). Pollen from a plant of the tester stock that was homozygous for a_1, sh_2, pr, and y had been used in making the cross to plant 6665E-9. Among the 169 Sh_2 kernels on the resulting ear, 1 was totally A_1 in phenotype, 38 were uniformly pale pigmented, 84 were fully variegated, 40 were colorless except for one or several A_1 dots, and 7 were totally colorless. If the pale colored class and the two latter classes are combined, the ratio of kernels types on this ear suggested that a fully active Spm might be carried in the Y bearing chromosome 6 of plant 6665E-9 because among the pale colored kernels and those with the modified phenotype, 30 were Y and 56 were y, and among the fully variegated class of kernels 45 were Y and 38 were y. In the former group, about one half of the kernels exhibited the modified

phenotype, being colorless or colorless with one or several A_1 dots. This suggested that some factor was present in plant 6665E-9 ~~whose presence~~ was responsible for modification of a_1^{m-1} expression, ~~that~~ it ~~was~~ segregated independently of Spm but could be recognized readily only when Spm was absent. It was decided, therefore, to examine plants derived from some of the colorless kernels that had only a few A_1 dots in them and these were grown in the summer of 1955 under culture number 6886. Spm-w was found to be present in ~~them, and again, it was located in chromosome 5~~.

On the testcross ear produced by plant 6662E-16, that was a_1 sh_2/ a_1 sh_2, Y/y in constitution (see table 18 for origin of this plant), some variegated kernels appeared that exhibited a much reduced number of A_1 dots in a colorless background in addition to ~~fully~~ variegated kernels. The pollen parent that was used in making the cross to plant 6662E-16 was homozygous for state 5718 a_1^{m-1}, for Sh_2, pr, y and wx and it had no Spm. The expected behavior in the presence of Spm of the a_1^{m-1} in this tester plant had been fully attested as it had been used in making many testcrosses to plants having Spm in them. On the ear produced by plant 6662E-16 there were 76 pale colored kernels, 26 of which were Y and 50 were y. In addition, there were 289 variegated kernels, 117 of which

were Y and 112 were y. However, the pattern of variegation among them was not the same. Some were fully variegated but others had a much reduced number of A_1 spots. Nineteen plants were grown from the fully variegated kernels on this ear (under culture number 6870 in the summer of 1955) and were each tested for the presence or absence of Spm-w in them in addition to the fully active Spm. From these tests conducted with these plants it was learned that a fully active Spm was located in the Y bearing chromosome 6 in plant 6662E-16 but in addition, an Spm-w element also was present and not linked with it. It appeared in 8 of the 19 plants derived from the fully variegated kernels on the ear of plant 6662E-16.

The Spm-w in plant 6683B-2

Discussion of Spm-w will commence with the plants in culture 6888, derived from kernels on the testcross ear of plant 6683B-2. As given above, this plant was a_1^{m-1} (state 5919A-1) Sh_2/a_1 sh_2, Pr/Pr, y/y, Wx/wx in constitution and the pollen parent used in making the cross with this plant had been homozygous for state 5719A-1 a_1^{m-1} and for Sh_2, pr, y and wx (see Table 1a) and it had no Spm. As reported, half of the kernels on this ear were uniformly pale pigmented and half were either colorless or had one to several dots of A_1 in a colorless background. Eight plants were grown from the uniformly pale class of kernels on this ear. Each was uniformly pale pigmented and ears of these plants were used in a cross with a plant that was homozygous for a_1, sh_2, and y and in which one fully

active Spm was present. This was done in xxxxxxxx order to learn whether or not the a_1^{m-1} in each plant would react normally to Spm. Ears were obtained from 7 of the 8 plants and the kernel types on these ears are entered in table 63. Half of the a_1^{m-1} carrying kernels on each ear were pale colored and half were variegated, giving the pattern of this expected when a fully active Spm element is present. Only two kernels on these 7 ears had a reduced number of A_1 dots in them. The kernel types on these ears contrasted greatly with the types appearing on the ears of plants that had been derived from colorless kernels in which only 1 or several A_1 dots were present when the same pollen parents were used in making crosses to them. The kernel types on these ears are given in table 64. Half of the a_1^{m-1} carrying kernels on these ears were fully variegated but among the remaining half, half of these, in turn, were xxxxx uniformly pale pigmented and half were either totally colorless or were colorless with 1 or several A_1 dots in them. Segregation of the alleles, xx Pr and pr, to the uniformly pale kernels and to the colorless kernels with 1 or several A_1 dots left little doubt that the factor responsible for the appearance of the colorless or nearly colorless kernels was carried in the Pr chromosome in these plants, and it was the chromosome that had been received from the parent plant, 6683B-2

The plants in culture 6888 ~~that gave on their~~ ears the kernels types were pale pigmented but ~~they~~ entered in table 64 ~~did not develop~~ pigment as rapidly ~~as~~ the plants that gave the kernel types entered in table 63. ~~The pigment developed much more slowly in them.~~ In some ~~plants,~~ the intensity of ~~this~~ was not uniform. ~~Sectors~~ appeared in which the intensity of this was much reduced or occasianally increased.

In addition to the crosses entered in tables 63 and 64, an ear was obtained from one plant in culture 6888 that had been derived from a ~~near~~ colorless kernel ~~as the result of a cross with a plant~~ homozygous for a_1^{m-1} (state 5718), and for Sh_2, pr, y, and wx and ~~in~~ had no Spm. On this ear there were 468 kernels; 253 were uniformly pale colored of which 70 were Pr and 183 were pr, 143 were colorless with 1 or several A_1 dots and 112 of these were Pr and 54 were pr, and in addition there were 69 totally colorless kernels. Again, it was clear that the ~~weakened~~ Spm in this plant was carried in the chromosome 5 that had been received from plant 6683B-2. Ten plants were grown from the uniformly pale colored kernels and ten other plants were grown from the colorless kernels with ~~0~~ or several dots of A_1 that were Pr in phenotype, under culture numbers 7264A and 7264B in the summer of 1956. All ten plants derived from the uniformly pale colored kernels were themselves, uniformly

pale pigmented. Those derived from the colorless kernels with few A_1 dots in them also were pale pigmented but ~~the extent of appearance of this pigment was much retarddd on the pigment~~ the pigment developed very slowly. Also, ~~some variation in~~ intensity of this ~~appeared within~~ some of these plants, ~~and in~~ distinct ~~sectors~~ regions within the plant. All plants in culture 7264 were crossed by plants that were homozygous for either state 5719A-1 a_1^{m-1} or state 5718 a_1^{m-1}, and for Sh_2, y, pr, and wx and that had no Spm in them. On ears produced by all plants in A of culture 7264, only uniformly pale pigmented kernels appeared. On all ears produced by plants in B of this culture, pale colored kernels, colorless kernels, and colorless kernels with one or several A_1 dots appeared. Linkage of the latter phenotypes with Pr and the ~~former~~ pale phenotyp with pr was clearly evident on all ears. The phenotypes of kernels on ears of 9 plants in culture 7264B are entered in line 4 of table 65. In one plant, 7264B-6, Spm-w was present and carried in the chromosome 5 with Pr but it was almost completely inactive in many kernels on this ear. The kernel types on this ear are entered in the last line of table 65. ~~The kernels~~ placed in the "inactive " ~~Spm-w~~ class were lightly pigmented and the distribution of this was uneven, giving a mottled appearance to the aleurone layer. This is ~~the~~ a typical appearance of the aleurone layer ~~of the~~ Spm ~~in a~~

is very weakly active.

Continued examination of the Spm-w, originally present in plant 6683B-2, included tests of its presence or absence in plants having a number of different constitutions. In order to facilitate presentation of the evidence obtained from these tests, several charts were constructed, figures 5 to 7. Figure 5 illustrates the origin of the plants in culture 6888 derived from kernels on the ear of plant 6683B-2, and the constitution of the pollen parent used in making a cross to each. From the ear of plant 6888D-2 (see 2, under Progeny Grown in Summer of 1955) kernels were selected and plants grown from them given culture number 7264 (see 3, under Progeny Grown in Summer of 1956). Kernels were also selected from both the first and the second ear on the main stalk of plant 6888C-3 (see under 1 of Progeny Grown in Summer of 1955) and the plants grown from them were given culture numbers 7262 and 7263. The phenotypes of the kernels selected from each of these two ears are given in figure 5 under 1 and 2 of Progeny Grown in Summer of 1956.

All plants in cultures 7262 to 7264 were crossed by plants that were ~~either~~ homozygous for ~~either state 5718 or state 5719A-1~~ a_1^{m-1} and for Sh_2, pr, y and wx and they had no Spm in them. On the ears produced by plants in culture 7262A and 7264A, no variegated kernels of any type

appeared. All were uniformly pale pigmented. Variegated kernels appeared on the testcross ears of all other plants in these three cultures. Each plant in cultures 7262C and D, in 7263C and D and in 7264B carried Spm-w but not Spm-s, and figure 6 was prepared to show the constitution of these plants with respect to the alleles of Pr, and the location of Spm-w. The kernel types on the ears of these plants are entered in table 65 according to the constitution given in figure 6. It may be seen that Spmw was carried in chromosome 5 in 22 of the 24 plants that were Pr/pr but in two plants, no evidence was given of linkage of it with this marker in chromosome 5.

The kernels that gave rise to the plants ~~having Spm-s in them (those~~ in B of culture 7262 and in A and B of culture 7263 ~~could not be Selected~~ for the presence or absence of Spm-w ~~in them nor~~ for the number of location of the Spm-s elements. In this respect, ~~they~~ proved to be quite heterogenous. Altogether there were 11 different constitution among the 21 tested plants, and these are given in figure 7. In 12 plants, no Spm-w was present. It was present in the remaining 9 plants and carried in chromosome 5 in each. The male parent of these plants had 1 Spm-s element in it, and it was located in one of its two y bearing chromosomes 6. Thirteen of the 21 plants in figure 7 were Y/y.

In 11 of them, one Spm-s was present and it was linked with y in 10 of these 11 plants. Two Spm-s elements were present in one plant (7263A-3, figure 7)one of which was carried in the y bearing chromosome 6. In the remaining plant that was Y/y (plant 7262B-2), three Spm-s elements were present, one of which was carried in its y bearing chromosome 6.

The types of kernels appearing on the ears of plants entered in figure 7 under the heading "No Spm-w" are given in table 66. They reveal the number of Spm-s elements present in each plant and the location of them with respect to the alleles of Y. In those plants that had an Spm-s element not linked with Y, no evidence was given of its linkage with either Pr in chromosome 5 or with Wx in chromosome 9. Also, on these ears, only one kernel was present that had a much reduced number of A_1 spots in its aleurone layer. The kernel types on the ears of plants entered in 1 and 2 under the heading "Spm-w present" in figure 7 are shown in table 67, and those entered in 3 and 4 under this heading are given in table 68. From the data ~~given~~ in each of these two tables, it is evident that Spm-s and Spm-w segregated independently of one another, and that in the majority of the Y/y plants, Spm-s was carried in chromosome 6, ~~and~~ Spm-w was carried in the chromosome 5 originally derived from the grandparent plant, 6683B-2. It also was clear that Spm-s is epistatic to Spm-w, all kernels

with Spm-s having many A_1 dots ~~in them~~ regardless of whether or not Spm-w is also present in them.

Response of state 5700A a_1^{m-1} to Spm-w

The following year, 1957, plants were grown from some of the kernels having Spm-w but no Spm-s in order to introduce Spm-w into plants having a state of a_1^{m-1} other than 5718 or 5719A-1. With either of these two states, the number of mutations to or towards A_1 is very much reduced when Spm-w is present, there being ~~xxxx~~ only 1/small A_1 dots, or several in some kernels or none in others. It was desired, therefore, to determine to what degree Spm-w would reduce mutation frequency with a state that gives a large number of mutant spots when Spm-s is present. For this test, state 5700A was selected (~~see photo~~. ~~00~~). State 5700A produced very many mutant areas both in plant and kernel with Spm-s and a number of mutations occur early in development of both plant and kernel. (all photo 00)

To obtain plants having state 5700A and no Spm in them, pale colored kernels were selected from the self-pollinated ear of plant 6702-2 which was a_1^{m-1}(state 5700A) Sh_2/a_1 sh_2, Pr/pr and it had no Spm in it (see table 18 and 23), and from the pale Sh_2 class of kernels on each of two ears produced by the cross of plant 6702-2 to plants that were homozygous for a_1 and sh_2 and in which no Spm was present. The plants having Spm-w,

grown in the summer of 1957, were derived from Spm-w carrying kernels on the ears of two plants, 7262C-2 and 7262D-4, entered in figure 6 and table 65. Each was Pr Spm-w/pr + and a_1^{m-1} (state 5719A-1) Sh_2/a_1 sh2 in constitution, and the ear of each was produced by a cross with a plant that was homozygous for state 5719A-1 a_1^{m-1} and for Sh_2, y, pr, and wx and in which no Spm was present. Plants were grown from kernels on each ear that had purple spots (Pr/pr) or red spots (pr/pr) in them. Tests were conducted with each plant that was used in a cross with a plant carrying state 5700A a_1^{m-1} in order to determine its constitution. For this purpose, pollen from plants homozygous for state 5719A-1 a_1^{m-1} and for sh_2, pr, y and wx and in which no Spm was present was placed on the silks of these plants. The kernel types on the resulting ears produced by 4 plants that were homozygous for state 5719A-1 a_1^{m-1} and Sh_2 and were Pr Spm-w/pr + are given in table 69. Spm-w was carried in the chromosome 5 with Pr in each of these plants.

Examination of kernel types appearing on the ears produced by intercrosses between plants carrying Spm-w and those having state 5700A indicated that state 5700A a_1^{m-1} responded to Spm-w by producing many fewer mutant spots than it does with Spm-s (see photos 00 and 00). Also, the time of occurrence of mutation was much delayed. Most of the A_1

spots in the kernels having state 5700A were small and this contrasted with the many spots of large size that appear when Spm-s is present. The number of pigmented spots was much greater, however, than that which appeared in kernels having either state 5718 or state 5719A-1 when Spm-w is present.

For further study of the effect of Spm-w on state 5700A a_1^{m-1}, kernels were selected from ears produced by plants that were homozygous for state 5700A in which no Spm was present when crossed by plants that were homozygous for state 5719A-1 and having Spm-w in them. All kernels on these ears had both state 5700A and state 5719A-1 in them. It was necessary to grow plants from kernels having both states of a_1^{m-1} in them and to cross them by plants that were homozygous for a_1 and in which no Spm was present in order to compare the response of each state to the Spm-w present in the plant. Among the kernels on the resulting ears, half should carry state 5719A-1 and the other half should carry state 5700A. The response of each state to Spm-w could then be compared directly among the kernels on an ear.

For one of the tests conducted ~~with~~ of state 5700 a_1^{m-1} to determine ~~its~~ the response to Spm-w, plants were grown from kernels on an ear produced by the cross of a plant that was Pr/pr and homozygous for state 5700A and Sh_2 and in which no Spm was present, by one that was homozygous for state 5719A-1, Sh_2 and pr and in which 1 Spm-w was present. Half of the kernels on this ear were pale colored and the other half were variegated. The pattern of A_1 spots among the variegated class of kernels resembled that produced by state 5719A-1 when Spm-s is present (see photos). Ten kernels were sown in the summer of 1958 from the pale Pr class of kernels under culture number 7530A and 10 other kernels were sown from the variegated, Pr class under culture number 7530B. All plants arising from the pale kernels were uniformly pigmented. Those arising from the variegated class showed small streaks of A_1 pigment in a non-pigmented background during early development and the pattern of streaks resembled that produced by state 5719A-1 in the presence of Spm-s. As the plants matured, pigment developed in the background. The pattern of small spots of A_1 in the kernels and of small streaks in the plant most probably were produced by mutation occurring to state 5700A, the contribution to these of state 5719A-1 being small. This was made evident by the kernel types that appeared on the testcross ears of these plants.

It was necessary to ~~learn~~ verify that the uniformly pigmented plants, arising from the pale class of kernels on the ear just described, had no Spm in them and also to verify that these plants carried state 5719A-1 in one chromosome 3 and state 5700A in the other chromosome 3, both of which would respond in the expected manner to the presence of Spm-s. Therefore, crosses were made to plants in culture 7530A using for this purpose pollen from plants that were homozygous for a_1 and sh_2. Some of the pollen came from plants that had no Spm in them whereas it was present in other plants. The types of kernels appearing on the ears of five plants in culture 7530A, produced by these crosses, are shown in table ~~69~~ 70.

It was ~~obvious~~ clear from these tests that no Spm was present in these plants and also that ~~the two~~ each states of a_1^{m-1} ~~present~~ in them would respond in the expected manner to Spm-s. ~~The contrast in the~~ Among the variegated kernels the pattern of variegation given by each state was so ~~great~~ distinctive that no difficulties were experienced in ~~separating~~ ~~the~~ kernels having ~~one or the other of these two states~~ state 5700A from those having state 5719A-1.

Plants in B of culture ~~that were variegated~~ were crossed by plants homozygous for a_1 and sh_2 and in which no Spm was present. Seven test cross ears were obtained from these plants and the types of kernels on each ear are given in table 70. All plants in culture 7530B were Pr/pr in constitution. It had been planned to cross these plants by ones that

were homozygous for a_1, sh_2, and pr but all plants of this constitution died in early stages of development due to unusually unfavorable growing conditions in the summer of 1958. Therefore, pollen was collected from plants ~~were used from~~ a culture in which the alleles of Pr were segregating. Some of the plants were Pr/Pr, others Pr/pr and still others pr/pr. The constitution of any one plant with regard to the alleles of Pr could not be known in advance of test of each. Of those used in the crosses to the plants in culture 7530B, all three constitutions were represented. It was expected that ~~the~~ Spm-w would be carried in the pr chromosome in these plants in culture 7530B and that linkage of the variegated class with pr would appear among the kernels on the testcross ears they produced. Among the ears given in table 7ϕ, one pollen parent was Pr/Pr, 4 were Pr/pr and 2 were pr/pr. It was evident that Spm-w was carried in the pr chromosome in each of the plants in culture 7530B entered in table 7ϕ. On the two ears produced by the cross with a plant that was homozygous for pr, the percent of recombinant kernels ~~with~~ among the variegated class was 33.

Among the variegated class of kernels on the ears entered in table 7ϕ two ~~classes~~ types could be distinguished readily. One had many dots of A_1 and the other had only one or several of them. In ~~the~~ total, there were 659 kernels of the former ~~class~~ type and 579 of the latter ~~class~~ type. In addition, there were 90 colorless kernels. If the colorless kernels are added to

to those in the latter class, a 1 : 1 ~~segregation~~ ratio appears among ~~for the~~ ~~two classes of~~ kernels having Spm-w in them. The latter class resembles in phenotype that appearing with state 5719A-1 and Spm-w and the former type in the same as that in the kernel which gave rise to each plant, and these kernels had both state 5700A and state 5719A-1 in them. Obviously, then, the kernels that have many A_1 dots carry in them state 5700A and those with one or several A_1 dots or just totally are colorless ~~with no A_1 dots~~, have state 5719A-1 in them. In other words, half of the kernels on these ears had no Spm-w in them and were uniformly pale colored and half carried Spm-w. Half of these, in turn, carried state 5700A and the other half carried state 5719A-1. It appears evident from these that Spm-w reduces mutation frequency in a proportional manner, the number of mutant spots that are produced in its presence being a fraction of that which appears when Spm-s is present. This relationship was also found to be true when Spm-w was introduced into plants carrying state 5996-4, which ~~produces~~ gives very many mutant spots with Spm-s but far fewer of them with Spm-w. Before this is considered, another test conducted with state 5700A and Spm-w will be discussed. It was ~~examined~~ made because ~~of~~ in the parent plant having Spm-w and this resulted in a change in action of Spm-w had occurred ~~that reduced~~ reduction in even further its capacity to induce mutation.

The ear of a plant homozygous for state 5719A-1 and Sh_2 that was Pr +/pr Spm-w was used in a cross with a plant homozygous for state 5700A, Sh_2, and Pr in which no Spm was present. On this ear there were 148 pale colored kernels and 140 colorless kernels with spots of A_1 in them. However, the number of A_1 spots was far fewer than that appearing on ears of other plants produced by similar crosses conducted with the plant carrying state 5700A. Instead of several hundred A_1 dots, the number among the varieg ted kernels on this ear ranged from as low as 15 to as high as approximately 100 with the majority of kernels having about 30 to 50 of them. Some of the variegated kernels were selected from this ear and grown in the summer of 1958 under culture number 7829. The ears of five of these plants were used in the cross with plants that were homozygous for a_1 and in which no Spm was present. The kernel types on these ears are shown in table 72 and the plants are arranged according to the proportion of kernels on their ears that were colorless with no A_1 spots in them. This was done because there was a distinct correlation between the percent of colorless kernels and these ears and the number of A_1 dots that were present in the kernels having state 5700A and Spm-w, the higher the proportion of totally colorless kernels on the ear, the lower the average number of A_1 dots that appeared in the kernels having state 5700A.

However, on all ears, a few kernels appeared with very many dots of A_1 in them or sectors appeared in some kernels in which the number of A_1 dots was very much increased. It appeared that the Spm-w in the parent plant that introduced it into the plants of culture 7529 was less effective in its capacity to induce mutation at a_1^{m-1} either with state 5700A or with state 5719A-1, and also that subsequent change in this was occurring in individual cells leading to a much increased capacity of it to induce mutation. Differences in mutation-inducing capacity of Spm as well as its inhibitory capacity to gene action at a_1^{m-1} have been noted in studies of other Spm-w isolates. Just as Spm-s may undergo change to give Spm-w type action, so may Spm-w undergo change to or toward Spm-s type action.

Change in degree of effectiveness of action of Spm-w may be noted in the plant as well as in the kernel. In some plants, distinct sectors appear in which the capacity of Spm-w to suppress gene action at a_1^{m-1} was increased. The anthocyanin pigment in these sectors was much lighter than in the rest of the plant. Also, some sectors appeared in which no pigment could be detected and within a few of them, small streaks of A_1 type pigment were present. From studies of the type described here and also from those that investigated inactivations of Spm, there seems to be little doubt

that the capacity of Spm to induce mutation is directly correlated with its capacity to inhibit gene expression at a_1^{m-1}. Spm-w represents and intermediate state between a fully active Spm and one that is totally inactive. Its capacity to induce mutation is reduced along with its capacity to inhibit gene expression. It is possible that the apparent double mode of action of Spm--supression of gene action at a_1^{m-1} and a_2^{m-1} and induction of mutation at both of these loci,--is the consequence of a single initial reaction in the cell for which Spm is responsible.

Another factor enetering into consideration of Spm-w will be mentioned here as it has significance in appreciating the relation of Spm-w to Spm-s. A modifier was found to be present in a single kernel on an ear of a plant carrying Spm-s. Its presence is made evident only when Spm also is present. The origin and detailed mode of behavior of it will be considered in the section of this report dealing with the Modifier. When present with either Spm-s or Spm-w, an approximate three-fold increase in mutation frequency is produced with states 5718 and 5719A-1 of a_1^{m-1}. The importance of mentioning it here is to indicate that the mutation increase it induces is the same with either Spm-s or Spm-w. Kernels having in them an Spm with a low grade of action can not be distinguished from ones in which the Spm is fully active whenever the Modifier also is present. However, if Spm is totally inactive, the Modifier has no effect

at all.

Response of state 5996-4 to Spm-w

Tests of the effect of Spm-w, derived initially from plant 6683B-2, on state 5996-4 a_1^{m-1} were conducted at the same time as those with state 5700A, just described. In thepresence of Spm-s, state 5996-4 gives an exceedingly high number of small mutant spots in both plant and kernel. The number of them may be so high that a kernel having this state and Spm-s often appears to be totally pigmented. Examination with magnification reveals that this phenotype is produced by the presence of many mutant spots located very close to one another. In some kernels that are purple, (Pr), the diffusion rims about each mutant spot may run together and this may make it difficult or impossible to know ~~with certainty if~~ from this examination alone whether the phenotype arose from a germinal mutation to A_1 or whether it was produced by many mutant spots whose ~~diffusi~~ diffusion rims have run together, making the aleurone layer appear to be totally A_1 in phenotype. From progeny tests, such a destruction could be made. In kernels that are homozygous for pr, however, the diffusion rims are much lighter in shade and in these kernels no such difficulties are experienced. The many dots, placed of A_1 although close to one another may be detected. ~~By use of~~ With the pr phenotype, it was learned that germinal mutation is infrequent with state 5996-4.

Because state 5996-4 produced many late occurring mutations with a fully active Spm-s, it is a very sensitive indicator of any somatically

occurring change in Spm. ~~and this applies to any state of Spm that may be present.~~ Sectors sometimes appear in kernels in which the action of Spm is altered. If the Spm present is undergoing change in a number of cells during development of the kernel, the pattern of mutant spots in the kernel is quite irregular. If Spm undergoes few changes during development the pattern is quite regular. Illustrations of this with Spm-s are shown in photographs 00 and 00. This state very effectively shows the types of change occurring to Spm-w. Spm-w, initially obtained from plant 6683B-2, reduces the percent of mutations with state 5996-4 as it did with state 5700A, and the mode of testing for this was the same as that just described for state 5700A. In one test, pollen from a plant that was a_1^{m-1} (state 5996-4) Sh_2/a_1 sh_2 was placed on the silks of a plant homozygous for state 5719A-1, and Sh_2 and having one Spm-w. On the resulting ear there were 196 pale colored kernels, 86 kernels with a number of A_1 dots in them that obviously had state 5996-4 in them, 98 kernels with 1 or several A_1 dots and 4 kernels that were totally colorless. The change in action occurring to Spm-w during the development of the kernels having it was illustrated in a striking manner by those that had state 5996-4 in them. In the majority of kernels, sectors were present in which many dots of A_1 were present and the pattern of this was similar

to that given when Spm-s is present. (See photos 00). In the kernels that had only state 5719A-1 in them, sectors appeared with more than the usual number of A_1 dots ~~were present~~ in them. It seemed clear that the Spm-w in the tributed by the female parent was undergoing rather frequent change in action or part during development of the kernel and this occurred early enough in some cells so that their progeny could ~~show~~ reveal this change ~~readily~~ by the altered pattern of A_1 dots in the sector each produced. ~~them.~~

When ~~the~~ pollen from the plant carrying state 5996-4 was placed on the silks of an ear of a plant bearing an ~~of another~~ Spm-w ~~carrying~~ of similar constitution to that just described, there were 167 uniformly pale pigmented kernels, 92 that had ~~a number of~~ dots of A_1 ~~in them~~, 39 that had only 1 or 2 A_1 dots and 62 that were totally colorless. It was obvious from the reduced number of A_1 dots and from the infrequent appearance of sectors in which many A_1 dots appe red, that the Spm-w contributed by the female parent was undergoing very few changes during early development of the kernel. Change in Spm-w was occurring but in individual cells but quite late in development. (See Photos 00). The impression gained from the study of state 5996-4 and Spm-w is that mutation to A_1 will occur with any state of a_1^{m-1} only after some modification has occurred to Spm itslef during development and that the number of mutant spots seen in a kernel with any one state is a reflection of the time of occurrence of change in Spm and the frequency of this occurrence at any one time.